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Spatial-temporal changes in dimethyl acetal (octadecanal) levels of *Octopus vulgaris* (Mollusca: Cephalopoda): relation to feeding ecology*

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SUMMARY: The present study aimed to identify and quantify the dimethyl acetal (DMA) levels in the muscle of *Octopus vulgaris* Cuvier, 1797, and to investigate the possible influence of octopus feeding ecology on their spatial and seasonal variations. The research was performed over one year in three areas of the Portuguese coast: Viana do Castelo, Cascais and Tavira. Significantly higher values of DMA were detected in Tavira ($p<0.05$), an area where the importance of bivalves in the octopus' diet was higher. The biochemical analyses performed in several prey items, belonging to five major taxonomic groups identified in octopus stomachs, revealed significant differences in DMA levels ($p<0.05$). The highest values were obtained in bivalves (1.15 mg 100 g^{-1} dry weight), followed by gastropods (0.74 mg 100g^{-1} dw), cephalopods (0.67 mg 100 g^{-1} dw), crustaceans (0.23 mg 100 g^{-1} dw) and osteichthyes (0.10 mg 100 g^{-1} dw). Therefore, based on these findings it can be speculated that the higher DMA levels in *O. vulgaris* obtained in Tavira throughout the year were evidence of its feeding ecology. Since DMAs are derived from ether glycerophospholipids, including plasmalogens (PLMs), the importance and role of this phospholipid class is discussed.

Key words: *Octopus vulgaris*, dimethyl acetal, plasmalogens, phospholipids, feeding ecology.

RESUMEN: CAMBIOS ESPACIO-TEMPORALES EN LOS NIVELES DE DIMETIL ACETAL (OCTADENAL) DE *OCTOPUS VULGARIS* (MOLLUSCA: CEPHALOPODA): RELACIÓN CON LA ECOLOGÍA TRÒFICA. – El presente trabajo ha tenido como objetivo la identificación y la cuantificación del nivel de dimetil acetal (DMA) en el músculo de *Octopus vulgaris* Cuvier, 1797, e investigar la influencia de la ecología alimentaria del pulpo en los variaciones espacio-temporales de este compuesto. El estudio ha sido realizado durante un año en tres zonas de la costa portuguesa: Viana do Castelo, Cascais y Tavira. Se han obtenido valores significativamente superiores del DMA ($p<0.05$) en Tavira, un área donde la importancia relativa de los bivalvos en la dieta fue superior. Los análisis bioquímicos realizados en distintos tipos de presas, pertenecientes a los cinco grupos taxonómicos identificados en los contenidos estomacales del pulpo, han revelado la existencia de diferencias significativas en los niveles de DMA entre los grupos ($p<0,05$). Los valores más elevados han sido obtenidos en los bivalvos (1.15 mg 100 g^{-1}), seguidos de los gasterópodos (0.74 mg 100 g^{-1}), cefalópodos (0.67 mg 100 g^{-1}), crustáceos (0.23 mg 100 g^{-1}) e osteichthies (0.10 mg 100 g^{-1}). En base a estos resultados se puede especular que los valores más elevados obtenidos en Tavira a lo largo de todo el año constituyen una evidencia de la ecología alimentar del pulpo. Dado que los DMA son derivados de los eter glicerofosfolípidos, donde se incluyen los plasmalogénos (PLM), se discute la importancia y el papel funcional de esta clase de fosfolípidos.

Palabras clave: *Octopus vulgaris*, dimetil acetal, plasmalógenos, fosfolípidos, ecología alimentaría.

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INTRODUCTION

In the animal kingdom, the main phospholipid classes are phosphatidylinositol (PI), phosphatidylserine (PS), phosphatidylethanolamine (PE), phosphatidylcholine (PC), cardiolipine (CL) and plasmalogens (PLMs) (Seewald and Eichinger, 1989; Drokin, 1993). PLMs, whose cellular functions have not been established yet (Zoeller *et al.*, 1999), are characterised by the presence of an aldehydic chain attached to a glycerol backbone through a vinyl ether (alkenyl) linkage (Malins and Varanasi, 1972; Horrocks and Sharma, 1980) and by a higher content of polyunsaturated fatty acids (PUFAs) than 1-alkyl-2-acyl and diacyl forms (Dembitsky, 1988). Their presence has been shown in a number of marine organisms (Rapport and Alonzo, 1960; Dembitsky, 1979; Sargent, 1989; Jeong *et al.*, 1999; Kikuchi *et al.*, 1999). It is also known that long-chain aldehydes are released from ether glycerophospholipids (including alkenylacyl glycerophospholipids – PLMs, and alkylacyl glycerophospholipids) on hydrolysis, and dimethyl acetals (DMAs) are formed from methylation of PLM aldehydes during esterification of phospholipids (Salih *et al.*, 1988). Consequently, many researchers have identified DMA as unknown peaks in fatty acid profile studies; this situation occurred in our previous study with *Octopus vulgaris* (Rosa *et al.*, 2002). Thus, the present work was initiated to confirm the identification of the DMA peak by mass spectrometry (GC/MS).

Octopus vulgaris has a world-wide distribution in temperate, subtropical, and tropical waters of the Atlantic, Indian and Pacific oceans; it is also present in the Mediterranean Sea (Mangold, 1998). It is a common and opportunistic predator on a wide variety of prey in many marine intertidal and subtidal communities (Altman 1967; Nigmatullin and Ostapenko, 1976; Guerra, 1978; Smale and Buchan, 1981; Ambrose and Nelson, 1983; Nixon, 1987; Sánchez and Obarti, 1993), and plays an important role in the food chain and in the ocean's ecology (Amaratunga, 1987; Boyle and Boletzky, 1996; Caddy and Rodhouse, 1998).

Though it is not known whether cephalopods have a limited ability to biosynthesise phospholipids *de novo*, like crustaceans (Shieh, 1969; Teshima, 1997), the influence of the lipid composition of the natural food on the body composition of cephalopods in both the muscle and the digestive gland has also been discussed by several authors (see Joseph, 1989). In fact, Navarro and Villanueva (2000, 2003) found a clear influence of the food in the fatty acid profile of the phospholipids of cultured octopus. Hence, the DMA levels may be related to the diet of *Octopus vulgaris*. Due to the lack of knowledge on this subject, the present study aimed to identify and quantify the DMA levels in *O. vulgaris* muscle and investigate the possible influence of octopus feeding ecology on the spatial and seasonal variations of DMA levels.

MATERIAL AND METHODS

Samples

The research was performed over a period of one year, beginning in January 2000 and concluding in January 2001. Twenty to thirty specimens of *Octopus vulgaris* were collected by commercial vessels (with traps and clay pots) with a monthly periodici-

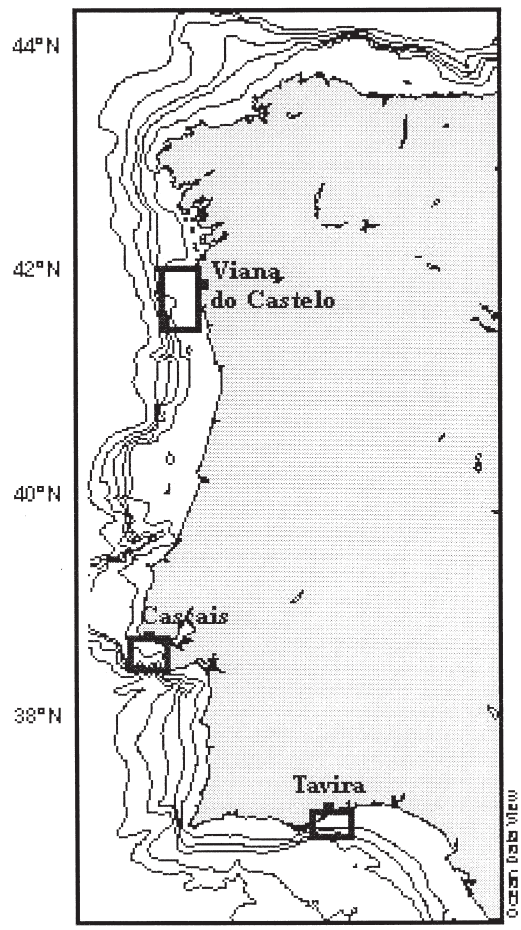

FIG. 1. – Map of the Portuguese coast with the *Octopus vulgaris* fishing areas of Viana do Castelo, Cascais and Tavira marked with a rectangle.

ty in three areas of the Portuguese coast: Viana do Castelo, Cascais and Tavira (Fig.1).

In each month, a sub-sample of five individuals was used and their muscle was pooled (homogenised) for the biochemical analyses. The total number of specimens analysed was 195. The total mantle length of the octopuses studied varied between 75 and 280 mm. In order to study the diet of *Octopus vulgaris*, the stomach contents were analysed in the three zones (402 octopuses in Viana do Castelo, 435 in Cascais and 453 in Tavira). The stomach contents were fixed in 5% formalin and preserved in 70% ethyl alcohol until further analysis. Prey remains were examined under a binocular microscope and identified to the lowest possible taxon. Fish remains include bones (usually vertebrae), otolith, scales, eye-lenses, skin and flesh. Crustaceans were rarely intact and remains usually consisted of fragments of exoskeleton, eyes or pinkish flesh. Cephalopod remains included beaks, arms, suckers, flesh and skin (with chromatophores). Gastropods and bivalves were recognised from flesh and shell remains. Polychaetes were recognised from jaws and chaetae. All the hard parts were identified after comparison with a reference collection and published descriptions (Zariquiey-Alvarez, 1968; Pérez-Gándaras, 1983; Clarke, 1986; Hayward and Ryland, 1998). For the diet description and comparison the occurrence index (OCI) was used, i.e. the number of stomachs with a specific type of prey expressed as a percentage of the total number of stomachs containing food (Hyslop, 1980). The vacuity coefficient (VC, the percentage of specimens with no food in their stomach) (Hyslop, 1980) was also used. In the present study, the genders were not taken into account, since previous studies in cephalopods have demonstrated no substantial differences in the diets of males and females (Guerra, 1978; Pierce *et al.*, 1994; Sánchez and Obarti, 1993; Quetglas *et al.*, 1999).

Between March 2001 and June 2001, both effective and potential (not referenced in the literature) prey items of *Octopus vulgaris*, included in five major taxonomic groups (Osteichthya, Crustacea, Cephalopoda, Gastropoda and Bivalvia), were collected on the Portuguese west and south coasts for the purpose of biochemical analyses.

GC/MS analyses and DMA quantification

As a consequence of the findings of our previous study, fatty acid methyl esters of *Octopus vulgaris* muscle were analysed by GC/MS in order to identify the unknown peak obtained in the fatty acid profile. The analysis was performed on a Kratos mass spectrometer, Model MS25RF, connected to a Carlo Erba HRGC-MS chromatograph, using a MASPEC II acquisition program. The separation was carried out in a fused silica capillary column (DB-Wax - 30 m x 0.25 mm; 0.25 µm film-thickness); the oven was programmed: i) to 40ºC and maintained for 1 min, ii) to 200ºC at 40ºC.min^{-1} and maintained at 200ºC for 3 min, iii) to 215ºC at 2.5ºC.min^{-1} and maintained at 215ºC for 2 min; iv) to 260ºC at 5ºC.min^{-1} and maintained for 6 min. Electron impact ionisation of 70 eV energy, with a mass range of m/z 35-400 and a cycle time of 1s (resolution 600) was used. The unknown compound was identified by comparison with the Wiley spectrum mass library.

The DMA content in the octopus muscle was determined in triplicate after transesterification, based on the experimental procedure of Lepage and Roy (1986) modified by Cohen *et al.* (1988). Methyl ethers and esters were analysed in a Varian 3400 gas chromatograph equipped with an auto-sampler and fitted with a flame ionisation detector. The separation was carried out with helium as the carrier gas in a Chrompack CPSil/88 fused silica capillary column (50 m x 0.32 mm id), programmed from 180ºC to 200ºC at 4ºC/min, held for 10 min at 200ºC and heated to 210ºC for 14.5 min, with a detector at 250ºC. A split injector (100:1) at 250ºC was used. Peak areas were determined using the Varian software.

Statistical analysis

Data were analysed using an ANOVA for comparison of multiple groups ($k \leq 3$). Previously, normality and homogeneity of variances were verified by Kolmogorov-Smirnov and Bartlett tests respectively. When data did not meet the assumptions of ANOVA, the nonparametric ANOVA equivalent (Kruskal-Wallis test) was performed. Having demonstrated a significant difference somewhere between the groups with the ANOVA and Kruskal-Wallis test, the Tukey Test and the Dunn Test respectively were applied to find out where those differences were. The *t* test or the Mann-Whitney test (the nonparametric *t* test equivalent) were applied for comparison of two groups ($k=2$) (Zar, 1996). The Statistica version 4.5 software was used.

TABLE 1. – Diet of *Octopus vulgaris* in Viana do Castelo, Cascais and Tavira. Occurrence index (OCI) of preys found in the octopuses' stomach contents.

Prey category	Occurrence index (OCI) Viana	Cascais	Tavira
ANNELIDA	1.85	2.52	-
POLYCHAETA unidentified	1.85	2.52	-
CRUSTACEA	38.27	67.31	36.90
ISOPODA unidentified	-	-	0.18
DECAPODA NATANTIA	1.93	6.04	2.57
Natantia unidentified	1.93	3.43	2.57
Caridae unidentified	-	2.61	-
DECAPODA REPTANTIA	36.34	61.27	34.14
ANOMURA	-	-	0.33
Paguridae unidentified	-	-	0.26
Pagurus prideaux	-	-	0.07
BRACHYURA	36.34	61.27	33.81
Brachiura unidentified	18.96	39.33	17.51
Portunidae unidentified	10.84	21.94	10.17
Liocarcinus sp.	5.17	-	5.03
Polybius henslowi	1.36	-	1.10
MOLLUSCA	7.13	14.89	23.52
GASTROPODA unidentified	1.49	1.87	0.61
BIVALVIA	1.75	7.43	16.56
Carditidae unidentified	-	-	1.07
Cardiidae unidentified	-	-	0.73
Mytilus sp.	-	-	1.72
Venus sp.	-	-	1.48
Bivalvia unidentified	1.75	7.43	11.56
CEPHALOPODA	3.90	5.59	6.35
Sepiolidae unidentified	-	-	1.39
Sepia sp.	-	-	0.73
Illex coindetii	0.71	-	-
Octopus sp.	0.18	0.83	0.36
Cephalopoda unidentified	3.01	4.75	3.87
OSTEICHTHYA	52.74	15.28	39.59
Clupeidae unidentified	16.70	3.29	12.94
Gobidae unidentified	5.80	4.53	1.43
Osteichthya unidentified	30.24	7.46	25.22

RESULTS

Diet

The OCI values for the prey items observed in the stomach contents of *Octopus vulgaris* from Viana do Castelo, Cascais and Tavira are shown in Table 1. Major taxonomic groups are summarised in Figure 2. In Viana do Castelo and Tavira, osteichthyes were the most common prey, appearing in 53 and 39% of the stomachs respectively. Crustaceans, namely Decapoda: Reptantia, constituted the second most important group in these two zones, with OCI of 36 and 34% respectively. The opposite situation occurred in Cascais, since Decapoda: Reptantia were represented in 61% of the stomachs, followed by osteichthyes with only 15%. Cephalopods also constituted an important group of prey (Viana: 4%, Cascais: 4% and Tavira: 6%), and the presence of octopodids in the stomachs suggested the occurrence of cannibalism or autophagy. Bivalves represented a higher percentage in Tavira (17%) than in Viana and Cascais (2% and 7% respectively). Finally, the diet of the species studied was completed by polychaete, gastropods and isopods, which appeared in low percentages (<3%) in the three zones analysed. With the exception of Cascais, the lower values for VC were obtained in the warm periods, i.e. in spring and summer (Figure 3) (Viana: $F_{3,9}$ = 13.83, Tukey Test, p<0.05; Cascais: $F_{3,9}$ = 36.12, Tukey Test, p<0.05; Tavira: $F_{3,9}$ = 24.54, Tukey Test, p<0.05).

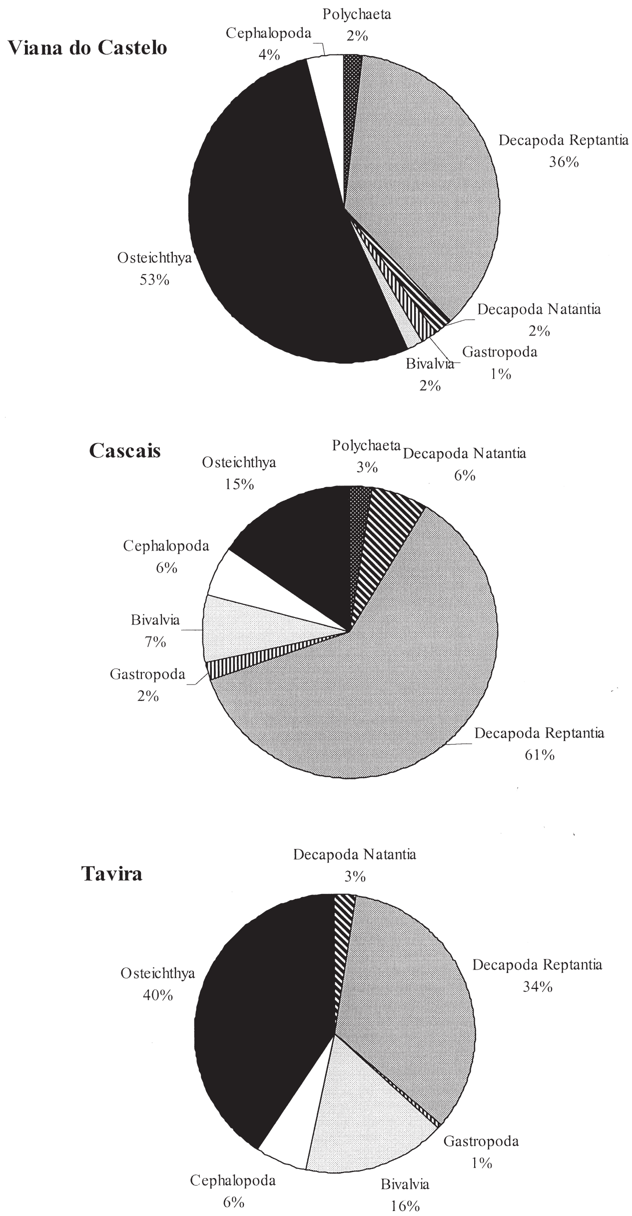

FIG. 2. – Percentage of the major taxonomic groups found in the stomach contents of *Octopus vulgaris* in Viana do Castelo, Cascais and Tavira.

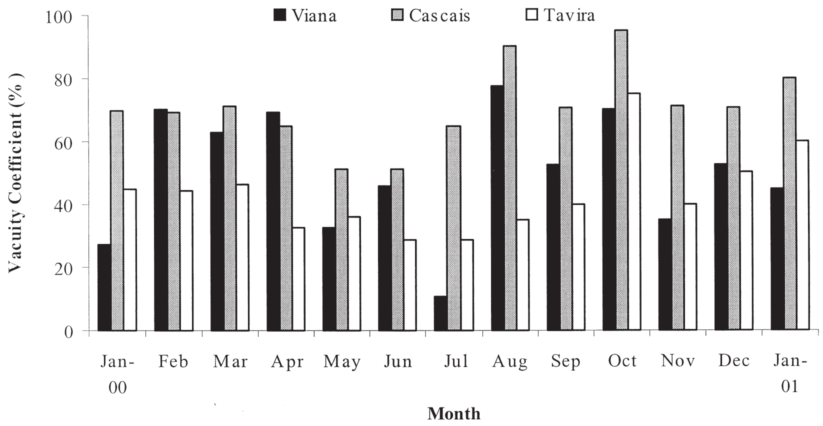

FIG. 3. – Seasonal changes in the vacuity coefficient (VC, %) of *Octopus vulgaris* stomachs in Viana do Castelo, Cascais and Tavira.

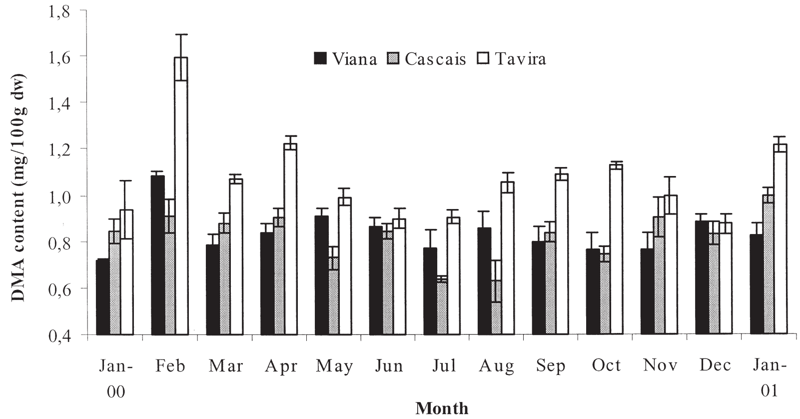

FIG. 4. – Seasonal variation in DMA of octadecanal content (mg/100g dry weight) in the muscle of *Octopus vulgaris* caught off in Tavira, Cascais and Viana do Castelo (monthly values in each zone are means of three replicates of a pooled sample ± SD).

Spatial and seasonal variations in DMA levels

GC/MS analysis indicated that the unknown compound obtained previously in the fatty acid profile had the mass spectra of DMA of octadecanal. Octadecanal ($C_{18}H_{36}O$) is an aldehyde with a molecular weight of 268.48; the molecular weight of DMA of octadecanal is 314.

The progression in the percentage of DMA of octadecanal in octopus muscle over the year (in the three zones of the Portuguese coast) is shown in Figure 4. Higher values were always obtained in Tavira (with the exception of December). In fact, significant differences were obtained between zones, especially in February ($H_2 = 7.20$, Dunn Test, $p<0.05$), April ($F_{2,6} = 89.01$, Tukey Test, $p<0.05$), October ($F_{2,6} = 64.53$, Tukey Test, $p<0.05$) and January 2001 ($F_{2,6} = 69.09$, Tukey Test, $p<0.05$). Significant seasonal variations were also attained in the three zones (Tavira: $F_{12,26} = 33.52$, Tukey Test, $p<0.05$; Cascais: $F_{12,26} = 12.00$, Tukey Test, $p<0.05$; Viana do Castelo: $F_{12,26} = 8.82$, Tukey Test, $p<0.05$), the highest values being obtained in February in Tavira and Viana do Castelo (1.6 and 1.1 mg $100g^{-1}$ dry weight respectively) and in January 2001 in Cascais (1.0 mg $100g^{-1}$ dw).

DMA levels in prey items

In order to investigate the possible influence of the diet on DMA levels in the muscle of *Octopus vulgaris*, DMA levels in the muscle of several effective or potential preys (belonging to Osteichthya, Cephalopoda, Gastropoda, Bivalvia and Crustacea) were also determined (Fig. 5). Significant differences were obtained between species ($F_{20,42} = 137.70$, $p<0.05$) (multiple comparison tests were summarised in Fig. 5). Comparing the five major taxonomic groups, the highest values were obtained in bivalves (mean group value: 1.15 mg $100g^{-1}$ dw; number of species=10), followed by gastropods (mean group value: 0.74 mg $100g^{-1}$ dw; number of

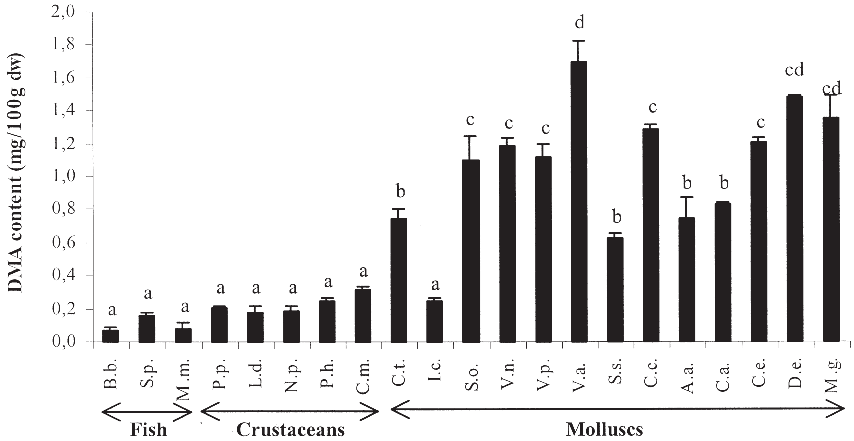

FIG. 5. – DMA of octadecanal content (mg/100g dry weight) of several effective or potential preys of *Octopus vulgaris*, belonging to: Osteichthya, B.b. *Boops Boops*; S.p. *Sardina pilchardus*; M.m. *Merluccius merluccius*. Crustaceans: P.p. *Pagurus prideaux*, L.d. *Liocarcinus depurator*. N.p. *Necora puber*; P.h. *Polybius henslowi*; C.m. *Carcinus maenas*. Cephalopoda: I.c. *Illex coindetii*; S.o. *Sepia officinalis*. Gastropoda: C.t. *Cassidaria tyrrhena*. Bivalvia: V.n. *Venus nux*; V.p. *Venerupis pullastra*; V.a. *Venerupis aurea*; S.s. *Spisula solida*; C.c. *Callista chione*; A.a. *Acanthocardia aculeata*; C.a. *Crassostrea angulata*; C.e. *Cerastoderma edule*; D.e. *Donisia exoleta*; M.g. *Mytilus galloprovincialis*. Values are means of three replicates of a pooled sample ± SD. Different letters represent statistically significant differences ($p<0.05$) between species.

species=1), cephalopods (mean group value: 0.67 mg $100g^{-1}$ dw; number of species=2), crustaceans (mean group value: 0.23 mg $100g^{-1}$ dw; number of species=5) and osteichthyes (mean group value: 0.10 mg $100g^{-1}$ dw; number of species=3). Among bivalves, the highest values were attained by *Venerupis aurea* (1.69 mg $100g^{-1}$ dw) and the lowest by *Spisula solida* (0.69 mg $100g^{-1}$ dw). Among cephalopods, it is worth noting that significant differences were obtained between the neritic and nektobenthonic species *Sepia officinalis* (1.1 mg $100g^{-1}$ dw) and the oceanic and demersal species *Illex coindetii* (0.24 mg $100g^{-1}$ dw) ($t_4 = 14.70$, $p<0.05$).

DISCUSSION

Feeding ecology

The versatile diet of the carnivore *Octopus vulgaris* is consistent with the general view of cephalopods as opportunistic predators and proves how adaptable they are (Summers, 1983). The higher values of fishes and crustaceans in the stomach contents could be related to their preference for these kinds of prey or to their availability in the octopuses' environment. It is worth noting that the results obtained from this benthic octopodid in Viana do Castelo and Tavira did not seem to corroborate the generalisation that benthic species tend to prey mainly on crustaceans while fishes predominate in the pelagic species (Castro and Guerra, 1990; Cortez *et al.*, 1995; Coelho *et al.*, 1997). In fact, according to Boyle (1990), the prey spectrum in the diet is related to the most readily available prey.

On the east coast of South Africa, Smale and Buchan (1981) showed that *Octopus vulgaris* had a greater preference for bivalves, mainly *Perna perna*, which formed up to 88% of their diet. Similar results were obtained by Ambrose and Nelson (1983) in French Mediterranean waters. These authors analysed the remains that were found near the areas inhabited by octopuses and reached the conclusion that molluscs made up 80% of their diet and the remaining 20% were crustaceans. In contrast, Altman (1967), Nigmatullin and Ostapenko (1976), Guerra (1978), Sánchez and Obarti (1993) and Quetglas *et al.* (1998) found a large proportion of crab and other crustaceans making up around 70% of the diet. In fact, it is known that data on the feeding habits of octopuses are biased by the sampling method used (Cortez *et al.*, 1995), and the proportion of bivalves and gastropods found in the stomachs might be underestimated, while in studies based on debris found near the middens they would be overestimated. Nevertheless, in the present study, a relevant spatial difference was obtained in relation to the proportion of bivalves in the stomachs, the highest values being attained in Tavira. In fact, the three zones studied have distinct bio-oceanographic and geomorphologic characteristics. The southeast coast of Portugal has mainly soft bottoms with sandy areas and mud (Marques, 1979; Moita, 1986;

Bettencourt, 1990), and the influence of the "Ria" Formosa system is also important due to the high levels of primary production (Sousa Reis, 1995). In Tavira, the common octopus lives in an environment with abundant and diverse bivalves, and consequently the importance of bivalves in the octopuses' diet was higher. Moreover, this could be one of the reasons, together with the higher average sea water temperature in this area, why octopuses from the southeast coast of Algarve have a higher growth rate than octopuses from other areas of Portugal (Sousa Reis, 1985), and could also explain some biochemical differences such as higher muscle protein levels (Rosa *et al*., 2002).

On the other hand, the Portuguese west continental shelf has mainly a hard rock bottom. The most important oceanographic characteristic is an upwelling, produced in summer when the winds from the north make deep water flow towards the shore, generating high primary production (Fiúza *et al.*, 1983). South of 40ºN, the shelf is deeply cut by submarine canyons that start near the coastline, which influence the oceanographic system in the area (Sousa Reis, 1995). These characteristics have an effect on the diversity, distribution and seasonality of the benthic, demersal and pelagic species, and consequently the opportunistic diet of *Octopus vulgaris* must be also influenced.

The high values of VC found in this study may be a consequence of the fast digestion of food, which characterises the common octopus (Boucaud-Camou *et al.*, 1976; Boucher-Rodoni and Mangold, 1977). The seasonal variations in feeding intensity were similar to Quetglas *et al.* (1998). VC was higher in winter (colder season). These findings are in agreement with the fact that cephalopods respond to temperature increases by increasing their food intake (Mangold and Boletzky, 1973).

DMA levels in *Octopus vulgaris* and prey

In view of the fact that some invertebrate marine organisms require dietary phospholipids for normal growth and survival (Shieh, 1969; Kanazawa *et al.*, 1985), the aldehyde composition of the alkenylacyl glycerophospholipids (PLMs) obtained in *Octopus vulgaris* may be a reflection of its dietary phospholipids. Since bivalves were the group of preys with the highest DMA values of octadecanal, and on the south Portuguese coast the importance of bivalves in the diet of *Octopus vulgaris* was higher, it seems likely that the higher DMA levels in *O. vulgaris* obtained in Tavira throughout the year were evidence of its feeding ecology.

If cephalopods are not so dependent on dietary phospholipids, and consequently the biosynthesis functions rapidly enough to satisfy their metabolic requirements, other explanations must be considered to justify the spatial differences in DMA levels. Based on the findings of Mourente and Tocher (1992), which found that the DMA levels increased from larvae to adults in the brain lipids from Atlantic herring, our results may be correlated with the size of the octopus specimens analysed. Though the size was not taken into account during sampling, it is known that the *Octopus vulgaris* from the Portuguese south coast have higher growth rates and attain higher weights than those from the west coast (Sousa Reis, 1985). On the other hand, Akulin (1975) found that the ratio of PLM to diacyl forms directly reflects the temperature of the organism's habitat. This could also be an explanation, since the south continental shelf is dominated by warmer sub-tropical waters, more specifically by a subtropical branch of Eastern North Atlantic Central Water (ENACW) (Fiúza *et al.*, 1998), and Mediterranean waters, originating in the outflow from the Strait of Gibraltar. The Mediterranean outflow runs below the 400 m isobath along the south coast and western coast up to Peniche (Ambar and Howe, 1979).

Although there is no reference in the literature associating the DMA or PLM levels with cephalopod dietary uptake, a direct relationship between the feeding ecology of *Octopus vulgaris* and DMA levels of octopus prey seems to be the most plausible explanation for our findings. Moreover, Joh and Hata (1979) revealed significant differences in the aldehyde composition of PLMs of seaweed-feeding gastropods and plankton feeding bivalves, and they were ascribed to the distinct modes of feeding. It is worth noting that the seasonal changes in DMA of *O. vulgaris* muscle did not seem to be linked with feeding intensity variations through the year as estimated with VC.

The aldehyde composition of PLMs in invertebrates is more varied than that of vertebrates, since it includes branched aldehydes and odd carbon number aldehydes (Jeong *et al.*, 1990, 1999). In vertebrates studies, Yamaguchi *et al.* (2000) found that the alkenyl chain composition of rainbow trout and carp plasma lipoproteins showed higher levels of hexadecanal (C16:0), followed by octadecenal (C18:1) and octadecanal (C18:0). On the other hand, carp brain PE showed C18:0 > C18:1 > C16:0 (Natarajan *et al.*, 1985). In other fish species, the C16:0, C18:0 and C18:1 alkenyl chains were the main components of the PLMs (Ohshima *et al.*, 1989; Medina *et al.*, 1993, 1995).

Several roles have been attributed to PLMs: antioxidants, terminal stores of arachidonic acid, receptors, mediators and determinant agents in the physico-chemical properties of biomembranes (Soudant *et al.*, 1995). However, none of these functions has been totally proved. It has also been suggested that PLMs are highly involved in the membrane permeability phenomena in response to environmental stress in marine ecology (Chapelle, 1987). In fact, the PLM form of PE showed a strong affinity for non-bilayer structure, a configuration that can exist locally during the fusion of the membranes or during the interactions of the membrane with external factors (Lohner *et al.*, 1991). Moreover, the PLMs in the PE content of the goldfish brain microsome increased as the O_2 concentration increased (Chang and Roots, 1985) and the PLM content of the mitochondrial membrane of the carp is reduced in the cold (Wodtke, 1981). The high PLM content (15.5-35.1% of total phospholipids) in crustacean gills is thought to play a role in ion transport processes as the gill is exposed to more oxidative stress than other tissues (Chapelle and Benson, 1986). More recent studies have demonstrated the selective degradation of PLMs when lipoproteins are exposed to reactive oxygen species (Engelmann *et al.*, 1994; Jungens, *et al.*, 1995), and PLMs may therefore function to protect cells against reactive oxygen species by mediating the damage (Zoeller *et al.*, 1999). Therefore, PLMs may be a component based on the environmental and physiological conditions, which could explain the distinct DMA levels obtained in fishes, crustaceans, cephalopods, gastropods and bivalves; they may be related to their different life histories. Moreover, Kostetskii and Sergeyuk (1986) established a relationship between the muscle PLM levels and the taxonomic position of several marine invertebrates.

For further study, the quantification by HPLC using acidic mobile phase, which allows a separate analysis of PLM forms of phospholipids and clarification of PLMs' role in the muscle of *Octopus vulgaris* is necessary. Furthermore, the relationship between feeding and PLM levels should be reviewed and fully investigated, since recent studies have only focused on PLM response to environmental stress in marine ecology.

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